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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Method for Producing Hybridoma Antibodies and Their Use

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ABSTRACT OF THE DISCLOSURE

A method is disclosed for producing hybridoma cell lines by fusion of immunized splenocytes of the mouse with mouse myeloma cells. The splenocytes are immunized against interleukin-6 prior to the fusion. Three different cell ines can be identified and isolated from the fused cells, that is the hybridoma cell line with the C.N.C.M. deposition number I/913 (BE-8), the hybridoma cell line with the C.N.C.M. depositon number I/911 (BE-4) and the hybridoma cell line with the C.N.C.M. deposition number I/912 (BF-6), which produce monoclonal antibodies which recognize human interleukin-6 and which in dependence upon the different cell lines each bind themselves to a different epitope on the interleukin-6 molecule.

The invention relates to the production of three new hybridoma cell lines and monoclonal antibodies produced therefrom which recognize human interleukin-6 or inhibit the action of interleukin-6.

The invention further relates to the use of these monoclonal antibodies for therapeutical and diagnostic purposes.

Interleukin-6 (IL-6), originally also referred to as human B-cell stimulation factor 2 (BSF-2) (M. Kawano, T. Hirano et al., Nature, Vol. 332, 3, 83-85, 1988) or Hybridoma Growth Factor (HGF) (R. Bazin and R. Lemieux, J. Immunol, 139, 78, 87, 1987), belongs to the mediators of the cellular immune system.

The sequence of the IL-6-coding cDNA and the amino acid sequence (184 amino acids) resulting therefrom is described in the literature (T. Hirano et al., Nature 324, 6, 73-76, 198 ....).

It is known that interleukin-6 (IL-6) has a wide spectrum of biological functions. The effects of interleukin-6 on T-cells (R. D. Gorman et al,. Proc. natn. Acad. Sci. USA 84, 7629-7633, 1987), plasma cytomes (J. van Damme et al., J. exp. Med. 165, 914-919, 1987); hepatocytes (T. Andus et al., FEBs Lett. 221, 18-22, 1987) and fibroblasts (M. Kohase et al., Cell 45, 659-666, 1986) are clearly described in the literature.

The role of IL-6 and IL-6 antagonists is described in the following general works:

"Regulation of the acute phase and immune responses"

"Interleukin-6",
Editor P.B. Sehgal et al.,
The New York Academy of Sciences, 2, East 63rd Street, New York, USA and
T. Andus et al., DMW 44, 1989

The above literature summarizes the significance of IL-6 in the formation of a great variety of syndromes and their courses and the influence by IL-6 antagonists (IL-6 antibodies) in hematological and solid tumours, in autoimmune diseases and inflammatory processes, in viral, bacterial and mycotic infections, in disturbances of the acute-phase reactions and in the influencing of the lymphokine cascade.

In addition, there are a great number of examples in the literature on the pathophysiological role of IL-6, of which only a few will be cited here by way of example.

Thus, C.P. Chin and F. Lee (J. of Immun., 142, 1909-1915, 1989) described the role of IL-6 in the regulation of growth and differentiation of myeloid leukemia cells.

P.A. Guerne et al. (J. Clin. Invest., 83, 585-592, 1989) show that particularly high levels of IL-6 were found in patients with inflammatory arthropathies and obviously play a significant part in the course of these syndromes.

C.E. Hack (Blood, 74, No. 15, 1704-1710, 1989) were the first to show that interleukin-6 plays an important part in the pathophysiology of septic shock. In their investigations they found that the majority of patients with septic shock exhibited greatly elevated IL-6 levels compared with the standard collective.

In investigations on the IL-6 level in patients with systemic lupus erythematosus, A.J.G. Swaak et al. (Rheumatol, Int. 8, 263-268, 1989) found correlations between the amount of IL-6 and acute-phase protein response. These and other investigations show that IL-6 is probably responsible for the induction of the production of acute-phase proteins by hepatocytes.

Kastleman's syndrome is a disease characterized by lymphocytic hyperplasia, plasma cell infiltration, fever, anemia and hypergammaglobulinemia. T. Nishimoto et al. (Blood 4, 1360-1367, 1989) were able to show that in this disease there is a clear correlation between the serum level of IL-6 and the clinical picture of the disease and the IL-6 must be regarded as the key element for the production of the various clinical symptoms.

The same working group around T. Kishimoto (FEBs Lett. 250, 607-610, 1989) were able to show that IL-6 is an autocrinic growth factor for renal cell carzinomas and that the growth of these tumour cells can be inhibited by an anti-IL-6 antiserum. The role of IL-6 as the decisive growth factor for myeloma cells has been investigated by a whole series of working groups:

X.G. Zhang et al. (Blood, 74, 11-13, 1989) were able to show that the IL-6 response of myeloma cells in vitro correlated directly with the proliferation status in vivo and thus with the gravity of the disease.

L. Bergin et al. (J. Exp. Med., 170, 613-618, 1989) were able to show that IL-6 not only acts on the malignant matured cell but that IL-6 promotes the proliferation and differentiation of malignant plasma cell precursors in multiple myelomas.

T. Kishimoto et al. (Nature, 332, 83-85, 1988) were able to prove experimentally for the first time that it is possible to inhibit the growth of IL-6-dependent myeloma cells with anti-IL-6 antibodies.

In PCT publication WO 88/00 206 the preparation and use of IL-6 is described and it is also pointed out that on using IL-6 in methods known per se polyclonal and monoclonal antibodies can be prepared. However, a way of preparing such antibodies is not described. Likewise, no antibody is defined by chemical/physical parameters and nor has a cell been deposited with which such an antibody can be produced. In this respect no reproducible teaching is given, i.e. the disclosure of this PCT publication is nothing more than purely speculative considerations.

The objective of this invention is to prepare monoclonal antibodies which are able to inhibit or suppress effectively the growth of IL-6-dependent cells. These monoclonal antibodies may be employed both therapeutically and also prophylactically in low doses so that no secondary effects occur during the treatment.

This objective is achieved by the use of the known fusion method, developed by C. Milstein and G. Köhler, and isolation of new hybridoma cell lines which produce mouse-monoclonal antibodies against IL-6.

From these cell lines with the designation BE-4 (IgG2b); BE-8 (IgG1) and BF-6 (IgG1), which are deposited at the French National Collection for Microorganisms (CNCM) under the numbers I/911, I/913, and I/912, class-switch variants of the mouse immunoglobulin may be isolated, such as for example IgG2a, IgG2b, IgG3, IgG1 and other immunoglobulin classes.

The monoclonal antibodies BE-4 and BE-8 compete with IL-6 for the binding to the IL-6 receptor on human and mouse cell lines and inhibit the proliferation of IL-6-dependent cell lines.

Both antibodies are able to recognize IL-6 which is bonded to the receptor and it has been shown that BE-4 and BE-8 recognize different epitopes on the IL-6 molecules.

The monoclonal antibody BF-6 likewise recognizes IL-6 but does not compete with IL-6 for the bond to the IL-6 receptor and nor is BF-6 able to inhibit the proliferation of IL-6-dependent cells. BF-6 is capable of recognizing IL-6 bonded to the receptor and it has been shown that it recognizes an epitope which is different from the epitopes recognized by BE-4 and BE-8.

The monoclonal antibodies are therefore suitable for the treatment of such diseases such as multiple myeloma, myeloid leukemia, Kastleman-syndrome, systemic lupus erythematosus, renal cell carzinomas, inflammatory arthropathy and other diseases which have been shown to be IL-6-dependent.

The monoclonal antibodies may be employed as pure substance, coupled to toxins (such as ricin A or saporine) or radioactive substances or other drugs, or encapsulated in liposomes.

Drugs containing the antibodies BE-4, BE-8 or BF-6 may be present in liquid or lyophilized form. For stabilization, proteins, sugars, sugar alcohols, amino acids and viscosity-enhancing agents may be used, and for buffering, inorganic salts, preferably Na phosphate in physiological medium (PBS pH 7.4).

The antibodies are used in a concentration of 0.5 5 mg/ml, preferably 1 mg/ml, for therapeutical purposes and as a rule administered systemically, although local administrations are not excluded.

The monoclonal antibodies BE-4, BE-8 and BF-6 may likewise be used as diagnostic reagents for identifying IL-6 at its receptor, on the surface of cells, or in body fluids. In such uses the antibodies may be coupled to fluorescent or other substances. It is likewise possible to use the antibodies for an ELISA or radioimmuno assay to measure IL-6 in body fluids.

The monoclonal antibodies in accordance with the invention are suitable for producing therefrom chimeric antibodies with the constant domain of human origin (human immunoglobulin) and the variable and in particular only the hypervariable region of murine origin (murine immunoglobulin). For the treatment of IL-6-dependent diseases the chimeras may be used as pure substance or alternatively coupled to toxins, radioactive substances, other drugs or encapsulated in liposomes.

The invention will be described in detail by the following examples:

I. Preparation of the monoclonal antibodies

Example 1: Immunization, fusion, cloning and recovery of the monoclonal antibodies BE-4, BE-8 and BF-6

Female Balb/c mice were immunized intraperitoneally 4 times at two-week intervals each time with 10 µg recombinant IL-6. The fourth immunization was administered intravenously and the splenocytes were extracted four days later and fused. The fusion was carried out as follows:

The immunized splenocytes were fused with X63Ag8653 murine myeloma cells in the ratio 5:1 in the presence of polyethylene glycol (Kearney et al., J. of Immunol. 123, 1548, 1978). This cell line is deposited at the European Collection of Animal Cell Cultures (ECACC), PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 0JG, UK under ECACC No. 850 114 20.

The fused cell suspension was washed once and cultivated in the selection medium (RPMI 1640, 10% heat-inactivated horse serum, 4 mM glutamine, hypoxanthine 13.6 mg/l, aminopterin 0.17 mg/l and 10 µg/ml insulin).

Ten days after the fusion supernatants of cultures which exhibited hybridoma growth were tested for anti-IL-6 mab production.

For this purpose 100 µl hybridoma supernatants was incubated for one hour in an ELISA plate which had previously been coated overnight at 4°C with 1000 ng anti-mouse immunoglobulin.

After washing three times the individual wells were each incubated for one hour at room temperature with 100 ng biotinylated IL-6 in 100 ml PBS. After washing three times again a reaction for one hour followed with streptavidin peroxidase at room temperature, another washing and incubation with substrate (DPO). Thereafter the plates were measured with an optical density of 405 nm. The positive clones were investigated after 4 cloning steps employing the limiting dilution method (seeding density 0.2 cells/culture) and the clones BE-4, BE-8 and BF-6 isolated. BE-4 is a mouse IgG2b antibody, BE-6 and BE-8 are IgG1 antibodies and all have a kappa light chain and exhibit a significant bonding to recombinant IL-6.

Example 2: In vivo production and purification of the antibodies BE-4, BE-8 and BF-6

The anti-IL-6 monoclonal antibodies BE-4, BE-8 and BF-6 were produced in large amounts in vivo by intraperitoneal injection of respectively BE-4, BE-8 and BF-6 hybridoma cells in Balb/c mice. One week prior to the hybridoma cell injection the mice were primed intraperitoneally with 0.5 ml Freund's incomplete adjuvant. It was possible to extract ascitic fluid 8-14 days after the hybridoma cell injection.

The mabs were thereafter precipitated from the ascitic fluid by ammonium sulfate (45 % saturation), rebuffered to 0.02 mM Tris, pH 7.7 and bonded to a Q-sepharose column. On these columns the mabs were washed with 1 % Tween 20 in 0.02 mM Tris, pH 7.7, and thereafter eluted from the column with 0.35 M NaCl solution, pH 7.7.

For therapeutical purposes the mab was rebuffered to physiological PBS buffer (phosphate-buffered saline).

II. Biological activity of the monoclonal antibodies BE-4, BE-8 and BF-6

Example 3: Inhibition of the IL-6-induced proliferation of the IL-6-dependent cell line B9 by BE-4, BE-8 and BF-6

The cell line B9 is a mouse hybridoma line which is dependent on IL-6 and the properties of which have been described by L.A. van Aarden (Eur. J. Immunol. 17, 1411, 1987). The cell line B9 is cultivated in RPMI1608 with

10 % fetal calf serum and mercaptoethanol with addition of 2 pg IL-6/ml for 3 days. Thereafter, for 16 hours H^3-thymidine is added. Thereafter the cells are collected, washed and measured in the beta counter.

In the following experiments, with the addition of 2 pg/ml IL-6 different concentrations of the antibodies BE-4, BE-8 and BF-6 were also added. The DNA synthesis was measured by determining the radioactivity as H^3-thymidine incorporation as a measure of the cell growth or cell inhibition.

Measured radioactivity (cpm)

		IL-6 (0 pg/ml)	IL-6 (2 pg/ml)
BE-4	0	6910	115976
	1 pg	7552	91601
	10 pg	7881	28247
	100 pg	7941	15384
	1 ng	9055	8326
	10 ng	8178	4860
	100 ng	7611	5327
	1 µg	6992	4315
BE-8	0	8916	136217
	1 pg	9002	64500
	10 pg	8225	28002
	100 pg	8264	16259
	1 ng	9027	8281
	10 ng	8356	5629
	100 ng	9221	5434
	1 µg	10836	7470
BF-6	0	7488	126167
	1 pg	8050	131178

10 pg	8366	116380
100 pg	9118	118153
1 ng	7673	124812
10 ng	7987	119631
100 ng	8855	115186
1 ng	9412	117318

cpm = disintegration per minute, mean value of two measurements.

The results clearly show that the H^3 thymidine incorporation and thus the DNA synthesis as a measure of the cell growth is appreciably lower than with B9 cultures tested with BE-4 and BE-8. These antibodies inhibit the IL-6-dependent growth of the cell line B9. BF-6 however is not able to inhibit the proliferation of the cell line B9.

Example 4: Scatchard Analysis: Determination of the affinity constant of IL-6 to the cell line U226

Iodination of IL-6:

5 µg IL-6 in 10 µl borate buffer (0.1 M, pH 8.0) was incubated with one mCi I^{125} (Bolton and Hunter Reagent of Amersham, Code IM 586) for 15 minutes at 0°C. The reaction was then stopped with 500 µl glycin (0.2 M in borate buffer 0.1 M pH 8.5) for 5 minutes.

Free I^{125} and bonded I^{125} were separated via a PD10 column (Pharmacia, G-25) which had previously been equilibriated with PBS 1 % bovine albumin. The specific activity of 1 ng IL-6 was 10320 cpm.

2.5 x 10^6 cells of the line U226/cup were incubated with various concentrations of I^{125}-IL-6 and 500 times excess of unmarked IL-6 for 90 minutes at 4°C in a total volume of 1

ml PBS 1 %. Washing was thereafter carried out three times and the measurements then made.

IL-6 µl	bonded IL-6 cpm	IL-6 ng	IL-6 total ng	free IL-6 ng	bon.IL-6 free IL-6
0.1	1900	0.01957	0.33	0.3104	0.063
0.2	3000	0.03914	0.66	0.6208	0.063
0.4	7500	0.07725	1.32	1.2427	0.063
1	16000	0.1640	3.33	3.166	0.052
2	29000	0.2987	6.66	6.3613	0.047
4	48000	0.4944	13.2	12.705	0.039
10	84000	0.8652	33	32.134	0.027
20	114000	1.1742	66	64.825	0.018
40	132000	1.3596	132	130.640	0.010

bonded IL-6 max.: 1.5 ng

0.057×10^{-12} mMol

$$kD: \frac{0.057 \times 10^{9}}{0.063} = 8.7 \times 10^{-10} \text{ M}$$

$$\text{Number of receptors/cell: } \frac{0.057 \times 10^{-12} \times 6 \times 10^{23}}{2.5 \times 10^{6}} = 13680$$

The Scatchard analysis shows a kD value of 8.7×10^{-10} M and 13680 receptors per cell with respect to the cell line U226.

Example 5: Receptor competition investigation between radioactively marked IL-6 and BE-4, BE-8 and BF-6

2.5×10^{6} cells U226 per well were incubated with 0.1 µl (0.33 ng) radioactively marked IL-6 and served as positive

control. The cpm count is 3269 ± 156 and was defined as value for the total bonding of IL-6.

On addition of 500 times excess of unmarked IL-6 to 0.1 µl (0.33 ng) radioactively marked IL-6, 253 cpm was found as value for the nonspecific bonding. This gives a value of 3016 cpm = 100 % for the specific bonding.

On carrying out the same experiment with radioactively marked IL-6 but with addition of 1 µg/ml BE-4, BE-8 or BF-6, the following values were obtained:

BE-4	:	11 % IL-6 bonding
BE-8	:	14 % IL-6 bonding
BF-6	:	92 % IL-6 bonding

This experiment clearly shows that BE-4 and BE-8 are able to inhibit the specific bonding of IL-6 to its receptor but that BF-6 is not able to inhibit this bonding.

Example 6: Scatchard analysis of the antibodies BE-4, BE-8 and BF-6

In each case 40 mg purified mab was incubated with Na^{125} I (0.5 mCi) in 180 µl PBS. Thereafter, 10 ml (0.4 mg/ml) chloramine T was added and the reaction stopped after 1 minute by adding 10 µl sodium bisulfite (0.5 µg/ml). The mabs thus marked were separated chromatographically (Sephadex-G-25) from free iodine.

For the Scatchard analysis I^{125}-BE-4, I^{125}-BE-8 and I^{125}-BF-6 were used in an ELISA:

- the plates were incubated with 1000 ng BE-4 or BE-8 or BF-6 per cup overnight at 4°C,

- thereafter saturation was carried out with PBS 5 % albumin,

- thereafter incubation was carried out with 10 ng IL-6 for two hours at 4°C,

- thereafter various concentrations of iodized antibodies and for certain concentrations also a success of unmarked antibodies were incubated for 90 min. at 4°C,

- 3 x wash and measure.

Measurements with U266 cell lines were carried out as follows:

Execution as in example 4 but instead of iodized IL-6 a constant amount of 20 ng IL-6 per 10^6 cells was incubated for 30 min. at 37°C and then various concentrations of iodized monoclonal antibodies added and incubated for 60 min. at 4°C.

Results:

specific activity:
BE-4 1 ng = 4664 cpm
BE-8 1 ng = 4571 cpm
BF-6 1 ng = 4510 cpm

Bonded mab	Marked tracer mab	kD	IL-6 molecules
BF-6	BE-4	0.8×10^{-9} M	15180
BE-4	BF-6	2.7×10^{-9} M	15168
BE-4	BE-8	0.1×10^{-9} M	14802

	UL266 IL-6 + antibody	U266 number of receptors
BE-4	3.9×10^{-9} M	11733

BF-6	4.7×10^{-9} M	27168
BE-8	2.5×10^{-9} M	13425

The ELISA Scatchard shows identical concentrations of IL-6 which was bonded in this system for all antibodies, i.e. the kD values found represent a reasonable value.

The kD values for IL-6 bonded to the receptor on the cell line U266 are different from the constant found in the ELISA. This can be explained by slight modifications of the three-dimensional structure of IL-6 after the bonding to the receptor.

The findings that BE-4 and BE-8 recognize less receptor-bonded IL-6 than BF-6 does can be explained in that BE-4 and BE-8 identify the active position of the molecule and that they can only recognize the IL-6 dimer at the receptor but that BF-6 can recognize the monomeric form apart from the dimeric form.

Example 7: Competition experiments between BE-4, BE-8 and BF-6

IL-6 (1 µg/cup) was bonded to the ELISA plates by incubation at 4°C overnight in carbonate buffer pH 9.5. Thereafter, the plates were saturated with PBS 5% albumin and washed four times. The respective radioactively marked BE-4, BE-8 and BF-6 was then added in different experiments and incubated. In addition, each radioactively marked antibody was also incubated with 1 x, 10 x, 100 x excess of unmarked antibodies.

	BE-4 I^{125} 8535 cpm			BE-8 I^{125} 9852 cpm			BF-6 I^{125} 6514 cpm		
BE-4	1x	10x	100x	1x	10x	100x	1x	10x	100x

unmarked	3597	1060	315	10517	9518	10468	6925	5621	6511
BE-8 unmarked	10810	8330	7077	4276	983	256	5974	4873	5035
BF-6 unmarked	8654	10448	8774	9518	8952	9328	3447	1130	703

This experiment shows that each antibody only competes with itself and there is no displacement between BE-4, BE-8 and BF-6. This means that all three antibodies have different epitopes.

Example 8: Sandwich ELISA for the measurement of IL-6

- Saturation with BE-8 (1000 ng/cup/100 µl) overnight at 4°C.
- Saturation with PBS 5 % albumin for 90 min. at room temperature.
- Various concentrations of IL-6 in human serum for 2 hrs at 37°C.
- Biotinylated BE-4 (6.5 µg/cup/100 µl, PBS, Tween 0.5%) for 90 min. at room temperature.
- Substrate, measurement

ng IL-6/cup/100 ml	O.D.
100	1.807
10	1.566
1	1.356

0.5	0.921
0.25	0.573
0.125	0.401
0.062	0.311
0.031	0.213
0.015	0.190
0.008	0.195
0.004	0.170

Background: O.D. 0.150

The sensitivity of the test is 0.3 ng/ml IL-6 in the serum.

Example 9: Inhibition of the IL-6-dependent human cell line GB by the antibodies BE-4 and BE-8

2 x 10^6 cells of the line GB per cup were incubated for 5 days at various concentrations of natural IL-6 and various concentrations of antibody BE-8 with addition of H^3-thymidine.

	cpm			
	0.15 µg/ml	0.08 µg/ml	0.04 lg/ml	0 µg/ml BE-8
IL-6 U/ml				
40	764	10213	56432	78421
20	119	110	7237	77610
4	222	280	594	73197
2	212	206	256	61040
1	196	224	230	38859

1 U IL-6: The amount of IL-6 necessary to induce 50 % maximum proliferation.

Identical results were obtained of the antibody BE-4. These results show a significant inhibition of natural IL-6 (from human lymphocytes) by BE-8 and BE-4 on the IL-6-dependent human cell line GB.

III. Initial clinical results with BE-4

Three patients with a diagnozed multiple myeloma in the final stage with increasing numbers of plasma cytoma cells, from 50,000 to 100,000 plasma cytoma cells per ml within 2 days, were treated with purified BE-4.

The dose was 10 mg per day over a total of 4 days (total dose: 40 mg). The antibody was infused in a concentration of 1 mg/ml in saline solution/1 % human albumin for 30 minutes. No side effects whatever were observed under the therapy.

Clinical findings:	The fever values rapidly dropped to normal body temperature. The serious feeling of illness in all patients disappeared immediately after the first administration.
Hematological findings:	The tumour mass (plasma cytoma cells) immediately diminished after the first infusion from 100,000 cells per ml to 40,000 cells per ml and remained stabile throughout the entire duration of the treatment.

The number of cells in the S-phase (predivision phase) dropped under the treatment from 30 % to 10 %.

These initial clinical results were obtained on patients who were in the final stage of the multiple myeloma

disease. It may therefore be expected that on treatment in an earlier stage of the disease the tumour mass can be diminished to such an extent that other treatment possibilities are opened up (for example bone marrow transplantation).

Since to this day no promising treatment scheme for these patients is available, the treatment with monoclonal antibodies against IL-6 will provide new stimulus for healing this fatal disease.

A further improvement will be obtained by a treatment with the antibodies BE-4 and BE-8 in combination, which recognize different epitopes and both inhibit the activity of IL-6.

Alternatively, the antibodies BE-4, BE-8 and BF-6 can be coupled to toxins which then make it possible, apart from the blocking of the IL-6 activity, to destroy the plasma cytomas by fixation of IL-6 to the receptor on the cells.

Claims

1. Method for producing hybridoma cell lines by fusion of immunized splenocytes of the mouse with mouse myeloma cells, wherein the splenocytes are immunized against interleukin-6 prior to the fusion and from the fused cells three different cell lines can be identified and isolated, that is the hybridoma cell line with the C.N.C.M. deposition number I/913 (BE-8), the hybridoma cell line with the C.N.C.M. deposition number I/911 (BE-4) and the hybridoma cell line with the C.N.C.M. deposition number I/912 (BF-6), which produce monoclonal antibodies which recognize human interleukin-6 and which in dependence upon the different cell lines each bind themselves to a different epitope on the interleukin-6 molecule.

2. Method according to claim 1, wherein the hybridoma cell lines are obtained with x63 Ag8653 murine myeloma cells.

3. Method according to claim 1, wherein the monoclonal antibodies have a specific bonding action to human interleukin-6.

4. Method according to claim 1, wherein the antibodies produced by the cell lines BE-4 and BE-8 can bind to the interleukin-6 receptor on human and murine cells.

5. Method according to claim 1, wherein the monoclonal antibodies form chimeras, the constant part consisting of human Ig and the variable, in particular the hypervariable part, consisting of murine Ig.

6. Method according to claim 1, wherein the monoclonal antibodies are coupled to toxins and/or chemothera-

peutical agents.

7. Use of the monoclonal antibodies obtained by the method according to claim 1 for therapy, profylaxis and diagnosis of interleukin-6-dependent diseases, in particular tumour diseases, autoimmune diseases, infections of any kind and disturbances of the acute-phase reactions.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente